REMARKS

Applicants would like to thank the Examiner for his careful reading of the subject application and his helpful suggestions for amending the claims.

Rejection of Claims 7, 20 and 21 under 35 U.S.C. §112, second paragraph

Claims 7, 20 and 21 are rejected under 35 U.S.C. §112, second paragraph "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention" (Office Action, page 3).

The Examiner states that there is insufficient antecedent basis for "said regulatory element" in Claim 7.

Claim 7 has been amended to replace the phrase "said regulatory element" with "said promoter", thereby obviating the rejection.

The Examiner states that Claims 20 and 21 "are indefinite because it is not clear whether the claims read on introduction of a retroviral vector into an animal" (Office Action, page 3). The Examiner states that the "rejection would be overcome by amending claim 20 to clearly read on cells in an animal or cultured cells" (Office Action, pages 3-4).

Claim 20 has been amended in accordance with the Examiner's suggestion.

Rejection of Claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 28, 29, 31 and 32 under 35 U.S.C. §103(a)

Claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 28, 29, 31 and 32 under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Faustinella et al." (Office Action, page 5). It is the Examiner's opinion that:

> [i]t would have been obvious to a person of ordinary skill in the art at the time the invention was made to modify the vectors of Couture et al. by adding the multiple cloning sites of Faustinella et al. because Faustinella et al. shows that multiple cloning sites may be used to insert sequences of choice in a U3 region of a retroviral vector (Office Action, pages 6-7).

In particular, the Examiner states that Couture *et al.* show "retroviral vectors comprising a substitution of a portion of the 3' U3 region with the corresponding region of 5 different murine retroviruses, including leukemia and sarcoma retroviruses", and that "after packaging, the substituted U3 region appears at the 5' LTR and serves as a promoter for all genes in the body of

the vector, and that different LTR constructs were preferentially expressed in specific cell types" (Office Action, page 6). The Examiner further states that Couture *et al.* show that "their chimeric LTR promoters are active in a cell type specific manner" and that "retroviral vectors with target cell specificity have utility in gene therapy protocols" (Office Action, page 6).

Applicants respectfully disagree. As pointed out in the Amendment A mailed to the U.S. Patent Office on September 16, 1998, Couture *et al.* constructed retroviral vectors "in which the U3 promoter/enhancer of Moloney murine leukemia (MoMLV) was replaced by the corresponding region from *five related murine retroviruses* (Couture *et al.*, abstract). Using retroviral vectors based on the Moloney murine leukemia virus, a "commonly used. . . gene delivery vehicle" (Couture *et al.*, page 667, column 1), Couture *et al.* proposed that "[v]ectors with the greatest expression level in a particular cell target could be built, or, alternatively, an expression level tailored to a particular trans-gene or disease by careful choice of the LTR" (Couture *et al.*, page 674, column 2). In the process of tailoring such MoMLV-based vectors, Couture *et al.* specifically chose "*minimally substituted murine retroviral vector LTRs*" (Couture *et al.*, page 674, column 2, emphasis added).

In contrast, Applicants' claimed invention relates to a retroviral vector which undergoes promoter conversion comprising in 5' to 3' order, a) a 5' long terminal repeat region of the structure U3-R-U5; b) one or more sequences selected from coding and non-coding sequences, said sequences being inserted into the body of the vector; and c) a 3' long terminal repeat region comprising a partially deleted U3 region wherein in said partially deleted U3 region a polylinker sequence containing a heterologous promoter *which is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based* is inserted, said promoter regulating, after infection of a target cell, expression of said one or more sequences selected from coding and non-coding sequences. In the specification as filed, Applicants show that a MLV-based retroviral vector carrying the unrelated promoter present in the MMTV LTR or the unrelated promoter present in the WAP LTR actively directed expression of a gene in the body of the vector and that the strict tissue specificity of each promoter was retained *in vivo* (*e.g.*, specification, page 23, line 24 - page 24, line 2).

Couture *et al.* do not teach or even suggest replacing the U3 promoter/enhancer of Moloney murine leukemia (MoMLV) with a heterologous promoter which is not derived from

the retrovirus or a related retrovirus upon which the retroviral vector is based. Rather, Couture *et al.* clearly direct the skilled person to replace a retroviral LTR with a "related" and "minimally substituted" LTR in order to achieve higher levels of gene expression in a specific cell type, and thus, teach away from Applicants' claimed invention.

Faustinella *et al.* do not provide the teaching that is lacking in the Couture *et al.* reference. Faustinella *et al.* teach a modified MLV vector comprising a partially deleted 3' U3 region which is substituted by either a luciferase reporter gene directly linked to a rous sarcoma virus promoter or a hygromycin resistance gene directly linked to a herpes simplex thymidine kinase promoter. Alternatively, Faustinella *et al.* teach that the gene directly linked to the promoter can be subcloned into the body of the vector. Faustinella *et al.* do not teach or even suggest that the promoter in the 3' U3 region of the MLV can be replaced with a heterologous promoter which is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based, for the purpose of directing expression of a coding sequence in the body of the vector (*i.e.*, directing the expression of a coding sequence which is not directly linked to the heterologous promoter in the retroviral vector).

An obviousness rejection requires both (1) that "the prior art would have suggested to the person of ordinary skill in the art that they should . . . carry out the claimed process"; and (2) that the prior art should establish a reasonable expectation of success. In re Vaeck, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). "Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in applicant's disclosure." Id. The cited art provides no motivation to combine their teachings, however, even if such a motivation were present in the cited art, the combined teachings would not render obvious Applicants' claimed invention.

At most, the combined teachings of Couture *et al.* and Faustinella *et al.* teach that:

1) if inserting a heterologous promoter directly linked to a gene to be expressed into a retroviral vector, then a heterologous promoter which is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based can be used to direct expression of the gene; and

2) if inserting a heterologous promoter that is not directly linked to a gene to be expressed into a retroviral vector (*e.g.*, when the heterologous promoter is inserted into the U3 region of the retroviral vector and the gene to be expressed is inserted into the body of the retroviral vector), then a *minimally substituted murine retroviral vector LTR* from the U3 region of a closely related retrovirus is to be used.

The combined teachings of the cited references do not teach a retroviral vector wherein the U3 region comprises a heterologous promoter *which is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based* and which regulates expression of a coding sequence inserted into the body of the vector after infection of the target cell. Furthermore, the combined teachings in the cited art do not provide a reasonable expectation that doing so would result in expression of the gene.

Furthermore, as pointed out in previously filed amendments in the instant application, at the time of Applicants' invention, the state of the art was such that one of skill in the art would not expect Applicants' claimed invention to be successful. (See, for example, pages 9-12 of he Amendment After Final mailed to the U.S. Patent Office on May 6, 1999 and entered in the instant application with the Continued Prosecution Application filed via Express Mail with the U.S. Patent Office on July 16, 1999 and page 9 of the Amendment mailed to the U.S. Patent Office on February 29, 2000.) Based on the general state of the retroviral vector art, after integration into the host cell genome transcription of the genes is expected in the Couture *et al.* constructs, but not in the constructs of the present invention. Couture *et al.* replaced the 5' U3 region with a corresponding region from closely related murine retroviruses. Accordingly, the 5' U3 region of Couture *et al.* is essentially unchanged. Thus, it is not surprising to the person skilled in the art that the 5' U3 regions in which only closely related regions and promoters are exchanged still allow transcriptional read-through of the 5' R region. However, in view of the arguments presented in the previously filed amendments, the skilled practitioner would not have considered inserting a heterologous promoter not related to the retroviral vector into a partially deleted U3 region for the purpose of directing expression of a foreign gene inserted into the body of the vector, because in this case the practitioner would have expected that a transcriptional read-through of the 5' R region, and, thus expression of the foreign gene would not occur.

Furthermore, in the scientific literature it is reported that genetic rearrangement occurs during reverse transcription, especially when heterologous elements are inserted into the U3 region of the LTR (see Junker, U., *et al., Gene Therapy, 2*:639-646 (1995) which was filed as the Exhibit with a previously filed amendment). Such genetic rearrangements are without relevance to the vector of Couture *et al.* because no heterologous elements are inserted into the U3 region. In the vector of Couture *et al.*, the U3 region is replaced by a minimally substituted U3 region from a closely related virus.

Clearly, the combined teachings of Couture *et al.* and Faustinella *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claims 13 and 14 under 35 U.S.C. §103(a)

Claims 13 and 14 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Faustinella et al. as applied to claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 29, 29, 31 and 32 above, and further as evidenced by Miller et al. and Panganiban et al." (Office Action, page 7). The Examiner states that Couture *et al.* in view of Faustinella *et al.* "do not explicitly show an altered retroviral gene or a partially deleted sequence involved in integration of retroviruses", but note that Couture *et al.* show that "their vectors are derivatives of the vectors of Miller et al. (Office Action, page 7). The Examiner cites Miller *et al.* as showing that "their vectors retain the phi+ packaging sequence, but lack the gag, pol, and env genes of a replication-competent virus"; and Panganiban *et al.* as showing that "the 3' end of the pol gene encodes the int locus that is required for integration of the reverse transcribed retroviral genome to form a provirus" (Office Action, page 7).

Applicants respectfully disagree. As pointed out above, the combined teachings of Couture *et al.* and Faustinella *et al.* do not render obvious Applicants' claimed invention. Miller *et al.* and Panganiban *et al.* do not provide the teaching lacking in Couture *et al.* and Faustinella *et al.* references. As discussed in the previously filed amendments, Miller *et al.* designed "a set of retroviral vectors which facilitate cDNA transfer and expression" (Miller *et al.*, page 986, column 3), one of which is the LNSX retroviral vector used by Couture *et al.* to generate their vectors. Panganiban *et al.* ('84) mutagenized cloned spleen necrosis virus and showed that the 3' end of the *pol* gene of the spleen necrosis virus encodes a polypeptide required for DNA

integration through interaction with the *att* site. Miller *et al.* nor Panganiban *et al.* do not teach or even suggest a retroviral vector wherein the U3 region comprises a heterologous promoter *which is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based* and which regulates expression of a coding sequence inserted into the body of the vector after infection of the target cell. Furthermore, Miller *et al.* and Panganiban *et al.* do not provide a reasonable expectation that doing so would result in expression of the gene.

Clearly, the combined teachings of Couture *et al.*, Faustinella *et al.*, Miller *et al.* and Panganiban *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claim 10 under 35 U.S.C. §103(a)

Claim 10 is rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Faustinella et al. as applied to claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 29, 29, 31 and 32 above, and further in view of Price et al." (Office Action, page 8). The Examiner states that Couture *et al.* in view of Faustinella *et al.* "does not show a vector derived from a BAG vector" (Office Action, page 8). The Examiner states that Price *et al.* show "a BAG retroviral vector comprising a beta galactosidase reporter gene, and that the vector can be used to identify cells and progeny of cells infected with the vector" (Office Action, page 8).

Applicants respectfully disagree. As pointed out above, the combined teachings of Couture *et al.* and Faustinella *et al.* do not render obvious Applicants' claimed invention. Price *et al.* do not provide the teaching lacking in Couture *et al.* and Faustinella *et al.* references. As discussed in the previously filed amendments, Price *et al.* applied a β-gal-transducing vector, BAG, "to the study of neural lineage *in vivo* and in culture" and were able to mark cells in both cases (Price *et al.*, page 158, column 2). In particular, Price *et al.* inserted the β-gal gene, the SV40 early promoter and the Tn5 *neo* gene into the body of the pDOL vector, which is derived from the Moloney murine leukemia virus (Mo-MuLV), and used the vector as a cell-lineage marking system applicable to the vertebrate nervous system. There is clearly no discussion in the Price *et al.* reference regarding the manipulation of the U3 region of the pDOL vector for any purpose.

Clearly, the combined teachings of Couture *et al.*, Faustinella *et al.*, and Price *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claims 15, 20, 21 and 26 under 35 U.S.C. §103(a)

Claims 15, 20, 21 and 26 are rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Faustinella et al. as applied to claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 29, 29, 31 and 32 above, and further in view of Longmore et al. and Kay et al." (Office Action, page 8). The Examiner states that Couture *et al.* in view of Faustinella *et al.* "does not show use of retroviral vectors in an animal" (Office Action, page 9). The Examiner states that Longmore *et al.* show that "mice infected with a retroviral vector expressing the erythropoietin receptor had increased platelet counts and splenic megakaryocytes"; and that Kay *et al.* show that "hemophiliac dogs infected with a retroviral vector expressing factor IX shows improved levels of clotting and thromboplastin times for greater than 5 months after treatment" (Office Action, page 9).

Applicants respectfully disagree. As pointed out above, the combined teachings of Couture *et al.* and Faustinella *et al.* do not render obvious Applicants' claimed invention. Longmore *et al.* and Kay *et al.* do not provide the teaching lacking in Couture *et al.* and Faustinella *et al.* references. As discussed in the previously filed amendments, Longmore *et al.* infected mice with a recombinant spleen focus-forming retrovirus (SFFV) expressing an oncogenic erythropoietin (Epo) receptor (EpoR) and showed a relationship between erythropoiesis and thrombopoiesis at the level of the Epo-EpoR signalling pathway. In addition, Longmore *et al.* teach that the SFV-based vectors "may be excellent vehicles for the introduction of genes into multipotent, hematopoietic progenitors, in vitro" (Longmore *et al.*, abstract). Using an amphotropic retroviral vector that encoded the canine factor IX complementary DNA, Kay *et al.* determined that a method for hepatic gene transfer *in vivo* by the direct infusion of recombinant retroviral vectors into the portal vasculature of a hemophilia B dog model, which results in the persistent expression of exogenous genes, may be feasible for the treatment of hemophilia B patients. There is no discussion in the Longmore *et al.* or Kay *et al.* references regarding the manipulation of the U3 region of their retroviral vectors for any purpose.

Clearly, the combined teachings of Couture *et al.*, Faustinella *et al.*, Longmore *et al.* and Kay *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

Rejection of Claim 7 under 35 U.S.C. §103(a)

Claim 7 is rejected under 35 U.S.C. §103(a) "as being unpatentable over Couture et al. in view of Faustinella et al. as applied to claims 1, 5, 8, 9, 11, 12, 15-19, 20-25, 29, 29, 31 and 32 above, and further in view of Mee et al." (Office Action, page 8). The Examiner states that Couture *et al.* in view of Faustinella *et al.* "does not show the claimed promoter or regulatory elements" (Office Action, page 10). The Examiner states that Mee *et al.* show "a retroviral vector comprising a mouse mammary tumor virus LTR, and that the LTR expressed a gene after induction with dexamethasone" and that "their vector is a potentially powerful tool for the manipulation of gene expression in a variety of cell types" (Office Action, page 10).

Applicants respectfully disagree. As pointed out above, the combined teachings of Couture *et al.* and Faustinella *et al.* do not render obvious Applicants' claimed invention. Mee *et al.* do not provide the teaching lacking in Couture *et al.* and Faustinella *et al.* references. As discussed in the previously filed amendments, Mee *et al.* teach the construction and properties of a self-inactivating (SIN) retroviral vector containing a hormonally regulated transcriptional element. In particular, Mee *et al.* disabled the 3' LTR of a retroviral vector and cloned the HRE inducible promoter of the MMTV and the *aph* gene directly between the LTRs of the provirus, *i.e.,* into the body of the vector (Mee *et al.*, pages 289-290). Mee *et al.* do not teach insertion of a heterologous promoter into a partially deleted U3 region of a retroviral vector.

Clearly, the combined teachings of Couture *et al.*, Faustinella *et al.* and Mee *et al.* do not render obvious Applicants' claimed invention, particularly as amended.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Anne J. Collins

Anne J. Collins
Registration No.40,564
Telephone (781) 861-6240
Facsimile (781) 861-9540

Lexington, Massachusetts 02421-4799

Dated: April 19, 2001

MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Twice amended) A retroviral vector which undergoes promoter conversion comprising in 5' to 3' order,

d) a 5' long terminal repeat region of the structure U3-R-U5;

e) one or more sequences selected from coding and non-coding sequences, said sequences being inserted into the body of the vector; and

f) a 3' long terminal repeat region comprising a partially deleted U3 region wherein in said partially deleted U3 region a polylinker sequence containing a heterologous promoter which is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based is inserted, said promoter regulating, after infection of a target cell, expression of said one or more sequences selected from coding and non-coding sequences.

7. (Twice amended) The retroviral vector according to Claim 31, wherein said [regulatory element] promoter is selected from the group consisting of Whey Acidic Protein specific regulatory elements and promoters, Mouse Mammary Tumor Virus specific regulatory elements and promoters, β-lactoglobulin and casein specific regulatory elements and promoters, pancreas specific regulatory elements and promoters, lymphocyte specific regulatory elements and promoters, Mouse MammaryTumor Virus specific regulatory elements and promoters conferring responsiveness to glucocorticoid hormones or directing expression to the mammary gland, and combinations thereof.

17. (Twice amended) A retroviral vector kit comprising:

a retroviral vector which undergoes promoter conversion comprising in 5' to 3' order, a) a 5' long terminal repeat region of the structure U3-R-U5; b) one or more sequences selected from coding and non-coding sequences, said sequences being inserted into the body of the vector; and c) a 3' long terminal repeat region comprising a partially deleted U3 region wherein in said partially deleted U3 region a polylinker sequence containing a heterologous promoter is

inserted, wherein said promoter is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based and said promoter regulating, after infection of a target cell, expression of said one or more sequences selected from coding and non-coding sequences; and

a packaging cell line harboring at least one retroviral or recombinant retroviral construct coding for proteins required for said retroviral vector to be packaged.

20. (Twice amended) A method for introducing homologous or heterologous nucleotide sequences into cells [of a human or] in an animal or cultured cells, said method comprising infecting the cells with recombinant retroviruses produced by the producer cell line of Claim 28.

28. (Twice amended) A producer cell line producing a retroviral particle, the producer cell comprising a retroviral vector and a DNA construct coding for proteins required for the retroviral vector to be packaged, said retroviral vector comprising in 5' to 3' order, a) a 5' long terminal repeat region of the structure U3-R-U5; b) one or more sequences selected from coding and non-coding sequences, said sequences being inserted into the body of the vector; and c) a 3' long terminal repeat region comprising a partially deleted U3 region wherein in said partially deleted U3 region a polylinker sequence containing a heterologous promoter is inserted, wherein said promoter is not derived from the retrovirus or a related retrovirus upon which the retroviral vector is based and said promoter regulating, after infection of a target cell, expression of said one or more sequences selected from coding and non-coding sequences.